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Studies in the Regeneration of Denervated Mammalian Muscle*

1. Volume Changes and Temperature Changes
2. Effect of Massage

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STUDIES IN THE REGENERATION OF DENERVATED MAMMALIAN MUSCLE.

I. VOLUME CHANGES AND TEMPERATURE CHANGES.

By F. A. Hartman, S/Sgt. W. E. Blatz and L. G. Kilborn.

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In conjunction with a study of the influence of treatment on denervated muscle we have carried out investigations concerning the circulatory changes which take place in it.

I. Volume Changes.

One of the immediate results which is known to occur after severing the nerve to a muscle is a dilatation of the blood vessels thus cut off from the nerve supply. This leads to an increase in the volume of the limb. We have studied these volume changes by means of the plethysmograph.

Methods.—Cats were the subjects for these experiments. They were anæsthetised with urethane except in one experiment where ether was employed. Volume changes were studied in one or both hind limbs, when both were used one served as a control. The limb was enclosed either in a glass or tin cylinder, which was made airtight by packing a vaseline-cotton-wool mixture between the thigh and cylinder mouth. The cylinder was connected to a Brodie bellows of such a capacity that it would be sure to accommodate the volume changes in the limb. The experiments were performed in a "constant" temperature room which was devised by the late Professor Brodie. Records were kept of the temperatures of both the room and air within the plethysmograph. Loss of heat from the plethysmograph was prevented by a thick wrapping of cotton-wool.

The sciatic and femoral nerves, in the limb to be denervated, were prepared for sectioning before fitting the plethysmographs in place. The former nerve was exposed just after it left the sciatic notch, and the latter where it emerged from under Poupart's ligament. A ligature was passed under the femoral nerve so that it could be lifted for cutting when desired. This method was not satisfactory in the case of the sciatic nerve because when the plethysmo-

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graph was in place the nerve could not be cut without pulling, nor could the plethysmograph be disturbed. In order to discover how much the stretching of the nerve might vitiate the results, the sciatic nerve was merely pulled after the limb had been enclosed in a plethysmograph. The volume change was almost as much as in a denervated limb. Of course the tension was greater than that which accompanies the preparation of the nerve for cutting. But it serves to demonstrate the care needed in preparing the nerves for cutting in these experiments. Therefore an instrument was devised which could be put in place so that the nerve could be cut at any time without moving the plethysmograph or stretching the nerve.

The neurotome (Fig. 1) is a small tube (*C*) plugged at the lower end (*a*). A hole (*b*) large enough to receive the nerve is cut in the

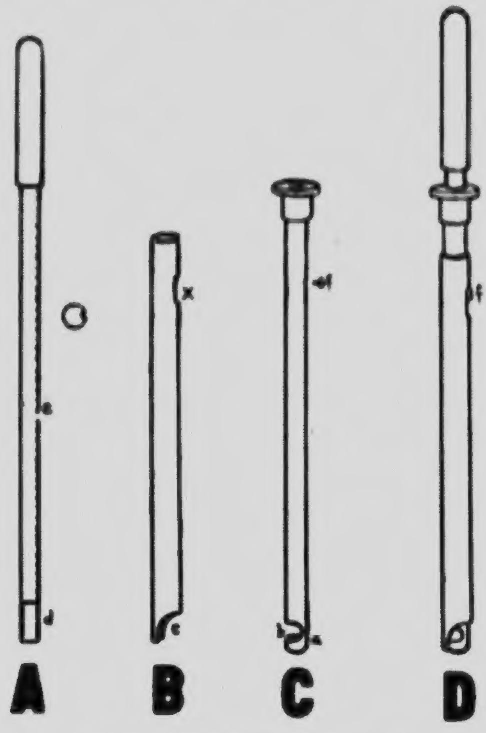

Fig. 1. Neurotome. (See Text.)

side. The escape of the nerve when in position is prevented by a well-fitting outer cylinder (*B*) with a projecting tongue (*c*), which is pushed into place from above (see *D*). The knife (*A*) is shaped like a chisel and is as wide as the internal diameter of the inner cylinder. This chisel (*d*) is fastened at the end of a brass rod (*e*) which fits snugly inside the inner tube. A bed for the chisel to work against is furnished by making a slot in the plug (*a*) at the lower end of the tube, to receive the cutting edge. A small screw (*f*) fastened in one side of tube (*C*) projects into a groove (*s*) in *A*. This prevents turning of the chisel. The same screw holds outer tube *B* in position, not only preventing rotation, but limiting its movement by slot *X*. (*D*) shows the instrument assembled.

After the nerves had been prepared for sectioning, the cylinder was put in place, and a record started on a slowly-moving kymograph. After the lapse of 30 to 60 minutes, when we were certain that the limb volume was constant, the nerves were cut. Records of the room temperature, rectal temperature, and of the air in the plethysmograph were taken every half hour in the early part of the experiment, and later every hour.

Results.—A cat under ether served as the first subject. Blood pressure from the carotid artery was registered every 30 minutes. The blood pressure fluctuated from 107 mm. to 128 mm. during the first five hours after denervation; then it gradually fell until it was 81 mm. at the seventh hour. The animal died at the end of eight hours. Maximum dilatation was reached five and one-half hours after denervation.

In a second cat under urethane the maximum dilatation was reached six hours after denervation. The blood pressure showed little change until the ninth hour where from a pressure of 92 mm. it gradually fell to 63 mm. by the end of the thirtieth hour. Unfortunately the temperature of the room was not controlled, so that a reduction in volume during the latter hours of the experiment cannot be taken as of any great significance.

Two more experiments were performed, in which changes only in the denervated limb were studied. In the first the maximum dilatation occurred 5·7 hrs. after denervation. This was one-half hour after the maximum temperature of the air within the plethysmograph was reached. These changes were independent of the room and rectal temperatures after the third hour. In the second animal, the maximum dilatation had developed at the end of the second hour. And then after an hour of little change, there was a fairly steady reduction in volume, especially pronounced during the succeeding six hours. This decrease in volume was too great to be accounted for

entirely by the decrease in rectal and plethysmographic temperature (see Fig. 2). By the end of the seventeenth hour the plethysmo-

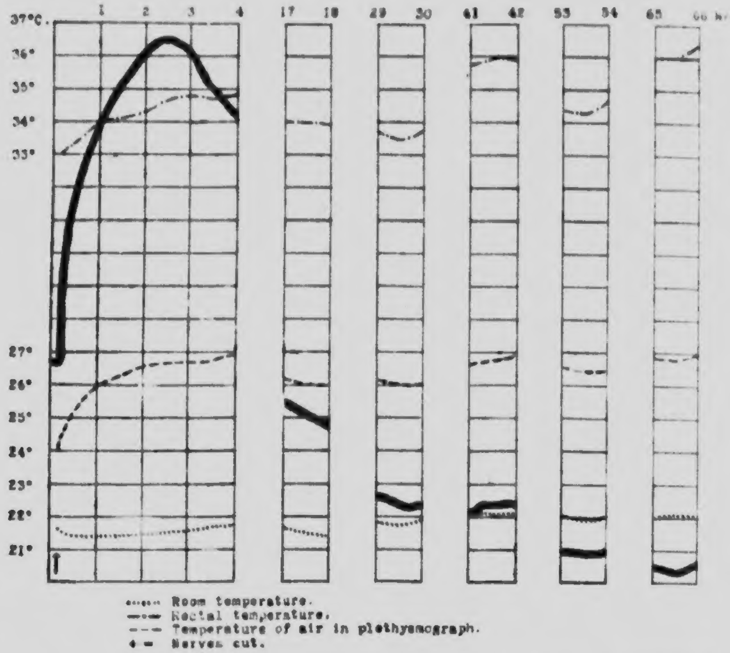

Fig. 2. Volume change in denervated hind limb. Heavy line represents limb volume.

graphic volume had returned to a little below normal. However, this was in part due to a fall in temperature of air within the plethysmograph. But by the forty-first hour there was a decided reduction in the volume of the limb which was independent of a reduction in air temperature within the plethysmograph because that temperature had again returned to its former height. This reduction of the limb volume continued to increase although the temperatures were not decreased. This experiment would seem to indicate that there is an over-recovery of the volume of the limb after dilatation and that this may be independent of a lowering of temperature in the limb. Indeed it appears that the limb temperature may remain as high as at the time of maximum dilatation. The bellows did not leak, and the animal remained in good condition, as indicated by the blood pressure,

until the experiment was stopped (75 hrs.). The blood pressure was 114 mm. at that time. The animal had recovered sufficiently from the urethane to give the corneal reflex.

In an attempt to secure better evidence of an over-recovery of the volume of the denervated limb, volume changes of both normal and denervated limbs were studied in three cats.

Unfortunately in the first experiment, the room temperature was allowed to drop after the seventh hour, so that constriction in the bellows took place as a direct result of this fall in temperature of the external air. However, the maximum dilatation had been reached at

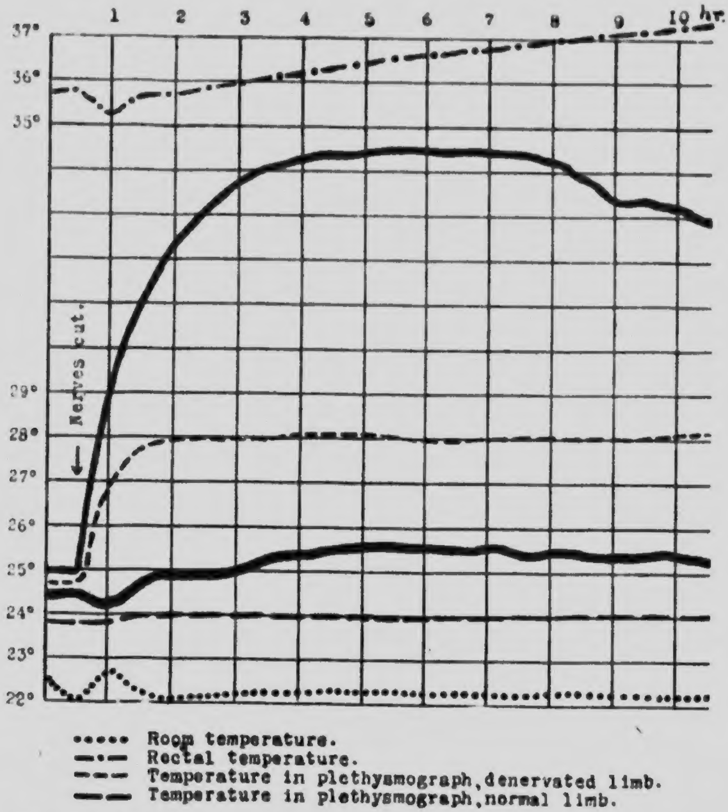

Fig. 3. Volume change in a denervated limb. Slower recovery than in Fig. 2. Upper heavy line, volume of denervated hind limb. Lower heavy line, volume of normal limb.

the fourth hour and the change was reversed within a few minutes, so that a steady constriction ensued. That this constriction was independent of any external influence, at least up to the ninth hour, was shown by the maintenance of the temperature of the air within the plethysmographs surrounding both limbs; moreover the normal limb did not constrict until the tenth hour. At the ninth hour the denervated limb had recovered its normal volume, as indicated by the bellows. Therefore this experiment tends to confirm the observation in the preceding experiment, that dilatation from denervation is recovered from in the course of several hours. The temperature within the plethysmograph surrounding the denervated limb did not begin to fall until four hours after the constriction had commenced. This also confirms the result in the previous experiment.

In the next experiment the room temperature was carefully controlled. Here again constriction of the denervated limb occurred unaccompanied by any fall of temperature of the limb as indicated by the air in the plethysmograph (Fig. 3). The maximum dilatation was reached in five hours, while the maximum temperature rise of the plethysmographic air had occurred in about two hours.

These results were further confirmed by a third experiment lasting twenty hours. In this, maximum dilatation was reached in two and one-half hours. From that time constriction took place, although so slowly that the normal volume had not been recovered until twenty hours after denervation. The temperature within the plethysmograph, however, did not fall until the fifteenth hour.

We next attempted to get some idea of the quantitative changes in the volume of a limb after denervation. In order to reduce the amount of air which might expand from the increased temperature of the limb, the cylinders were filled with cotton-seed oil after being carefully fitted to the limbs, merely the tube and bellows and a small space near the opening of the tube containing air. At the end of the experiment, immediately after the death of the animal, the temperature of the oil was raised by external heat, then by noting the temperature increase, together with the bellows changes, we could tell approximately the volume increase for each increase of a degree. By subtracting the expansion which would be due to rise of temperature of the air in the plethysmograph during the experiment the volume increase due to expansion of the limb was estimated. According to this method the limb actually expanded 2·7 c.c. The volume of the limb was determined by displacing water from a cylinder. This method of course could not be accurate, but it gave a fair idea of the magnitude involved. The denervated limb was found in this manner to be 105 c.c. in volume. The percentage expansion then was about 2·5.

Discussion.

Gaskell (1) reached the conclusion that the increased blood flow due to denervation was very transient in character. He studied the venous flow from the extensor vein in dogs. The maximum increase in flow was attained in 20 to 40 seconds. The increase disappeared in from two to four minutes, and it amounted to as much as nine to eighteen times the normal. He showed that the quick recovery to the normal rate of flow was not due to loss of blood, because if he waited for a few minutes after cutting the nerve before the blood was permitted to escape, the rate of flow was normal.

Goltz (2) believed that the dilatation resulting from nerve section was due to excitation of vasodilators. If that is correct rather quick recovery of the vessels should be expected as the effect of stimulation would not last long.

Our observations are based on the volume change which may or may not indicate a change in rate of flow. In view of Gaskell's work the most plausible explanation of our result is that following the transient dilatation and resulting increase in flow, there is constriction at some region such as the arterioles, capillaries or venules, while the other vessels continue to dilate or remain in an expanded condition. Thus the rate of flow might be quickly reduced to normal, but due to the congestion the limb remains dilated. Then as time went on the congested vessels gradually recovered their former size, so that in the course of many hours the congestion or dilatation would disappear. If this is the correct interpretation, the increase in volume does not indicate an increased circulation to the limb. In fact the rate of flow might actually decrease.

On the other hand such an interpretation would not account for the prolonged rise in temperature which has been observed by many investigators (2, 3). This must be due to either increased circulation or else to a local increase in heat production. As far as we know, heat production in muscle is associated with contraction. Therefore increase in tone or contraction would be necessary to account for such a condition. The only indications of increased muscular activity are the observations by Schiff (4) and others that fibrillation occurs in denervated muscle. However, Langley, and Kato (5) did not observe fibrillation until four days after nerve section. This makes a considerable gap during which fibrillation apparently could not account for a local temperature increase.

The increase in volume together with the increase in temperature seem to indicate a prolonged circulatory increase after nerve section. We are unable at present to reconcile this view with Gaskell's work.

II. Temperature Changes.

It is well known that the temperature of a muscle is increased after denervation. Goltz (2) studied the change in skin temperature in a denervated limb. He found that the increased temperature persisted from ten to twenty-eight days. His method, however, did not permit very exact observations. Heidenhain (3) observed the temperature of denervated muscle by means of a very sensitive thermo-electric couple. He came to the conclusion that the rise of temperature often lasted for weeks. Many others (1, 6) have found similar results.

We have tried to follow the temperature changes over longer periods of time than was done by the earlier workers. For example, where they made observations continuously for but a few hours after denervation, we have done so for two or three days in some cases.

Methods.—Cats were used as in the volume experiments. They were placed under the influence of urethane in experiments of long duration, but when only one set of readings was desired ether was employed. The animal was protected from loss of heat by covering with cotton-wool and by carrying on the experiment in an unusually warm room. Sometimes artificial heat was supplied by means of an electric heater arranged to reflect upon the animal. Many of the experiments were conducted in a "constant" temperature room. Artificial heat was furnished by 8 c.p. carbon lamps located in different parts of the room near the floor. These lamps were turned on or off as the temperature showed a tendency to vary. The room was ventilated by an electric fan. Undue loss of heat from opening the door was prevented by a vestibule and second door.

The femoral and sciatic nerves were exposed and cut with a neurotome as described in § I.

We used very sensitive mercury thermometers which registered from—5° to 50° C. They were graduated in tenths of a degree and each division was great enough (each degree was 6·5 mm.) so that hundredths could be estimated. Readings were made by means of a lens. These thermometers had been carefully compared with each other at temperatures ranging from 18° C. to 40° C., so that all readings could be corrected.

In order to determine the temperature within the limb, thermometers were inserted through slits in the skin between the muscles and allowed to remain there throughout the experiment. One thermometer ("distal") was inserted next to the inner surface of the gastrocnemius by passing it through the skin at one side of the tendon of Achilles and then back well up under the belly of the muscle. For the other thermometer ("proximal") an opening was made in

the skin opposite the popliteal fossa. Through this the thermometer was passed upward and underneath the thigh muscles. Both hind limbs were thus supplied with thermometers. The rectal thermometer was inserted as deeply as possible into the rectum. Although one limb served as a control, the temperatures in the limb to be denervated were determined over a period of at least one hour preceding the cutting of the nerves. The thermometers were not changed in position, once the experiment was started.

Temperature increase in a denervated limb.—The first experiment ran for ten hours following the denervation. The temperature of the denervated limb increased rapidly for ten minutes following the cutting of the nerves and then slowly during the remainder of one and one-half hours, after which they maintained a course parallel with those in the normal limb, but from 0·4° to 0·5° C. higher. The four thermometers in the limbs showed an increase in proportion to the increase in rectal temperature which developed during the experiment. The different temperatures at different stages of the experiment were as follows:—

	Just Before Denervation.	1·3 Hrs. after Denervation.	2·5 Hrs. after Denervation.	3·5 Hrs. after Denervation.	9·7 Hrs. after Denervation.
Rectal	32·83°C.	33·80°C.	34·65°C.	35·30°C.	36·06°C
Denervated "proximal" limb.	31·80	33·40	34·00	34·60	35·25
Normal "proximal"	31·70	32·90	33·55	34·15	34·75
Denervated "distal" limb	30·15	32·62	33·3	34·0	34·20
Normal "distal" limb	30·40	31·83	32·8	33·7	33·4

In a second experiment of this kind the thermometers had been sterilized and aseptic precautions were observed in inserting them beneath the muscles. The temperatures (Fig. 4) were taken at 10 minute intervals for 50 minutes preceding the sectioning of the nerves. Afterwards they were read at intervals of at first five minutes, then fifteen minutes, later thirty minutes, and finally one hour and two hours in length. The temperatures were registered for 60 hours after cutting the nerves. The animal appeared to be in good condition up to the forty-fourth hour, and remained under the influence of urethane. At that time a fever developed, due to tracheal infection. It died from occlusion of the trachea by mucous and pus at about the sixty-third hour. Throughout the experiment the temperature of the normal limb tended to keep parallel with the rectal temperature, but from 0·5° to 0·9° C. below. This difference became

greatest when the room temperature was lowest, especially in the region of the distal thermometer. On the other hand the temperature of the denervated limb increased 0·4° C. during the first ten minutes after cutting the nerves. This increase became gradually greater during the first hour until it was about 0·5° C. more than the normal limb.

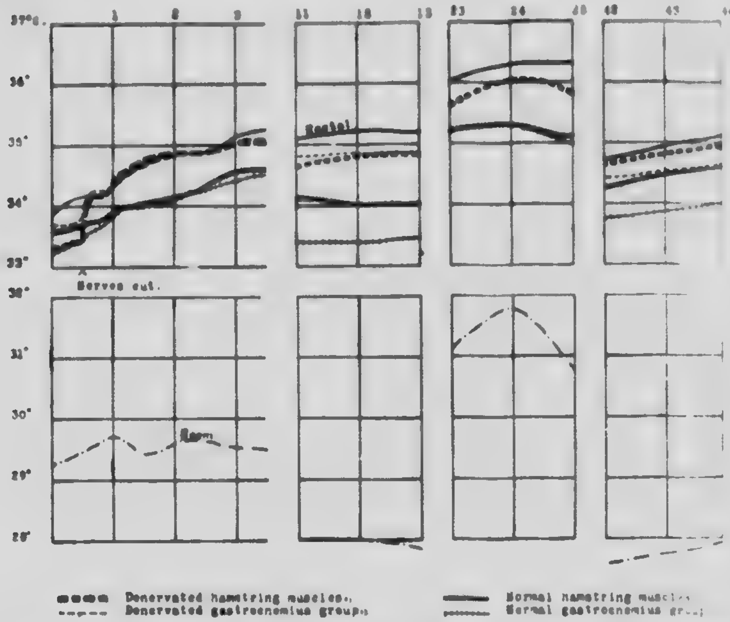

Fig. 4. A comparison of temperatures within the normal and denervated hind limbs of a cat. Shows the effect of varying the room temperature upon "distal" and "proximal" temperatures in the normal and denervated limbs. Time, hours.

There was some fluctuation in the temperature difference between the normal and denervated limbs, it being greatest when the general body temperature (as determined by rectum) was lowered by allowing the room temperature to fall. Then the difference between the proximal thermometers became as great as 0·9° C. while that between the distal thermometers was as large as 1·5° C. The temperature difference became least when the general body temperature was greatly raised. Then the proximal and distal thermometers registered nearly the same, while the difference in temperature between the normal and denervated limbs became as small as 0·25° C.

In a third cat similar changes in temperature were produced (Fig. 9). There was a decrease of 0·45° in the normal limb, following denervation of the opposite limb, perhaps due to general vascular constriction, which takes place to counteract the dilator effects of denervation on general blood pressure. As before, the temperature of the denervated limb, although it soon exceeded the rectal temperature by about 0·2°, kept very near the rectal for several hours. Between the fifth and sixth hours the rectal temperature became less than that of the denervated limb. And in twenty-four hours the latter had decreased so much that the difference between the two was 0·7° to 0·8°. Changes in the room temperature influenced the temperature of the normal limb, as was to be expected, but had little or no effect upon that in the denervated limb.

Temperature decrease in a denervated limb.—Having determined the temperature increase, we next proceeded to find out the limits of temperature decrease in a denervated limb. This is of interest in connection with treatment, especially massage, of denervated muscle. The decrease in temperature may be due in part to constriction of the blood vessels. In such a condition massage might assist the circulation at least temporarily.

The time of "over-recovery" of a denervated limb is of considerable variation in different individuals. In order to discover when the "over-recovery" had taken place, the relative temperatures of the paws of the denervated and normal limbs was determined every few days by a method which Dale and Richards describe (7). The paw was immersed in a test tube containing 10 c.c. of water at room temperature. Movement of the paw stirred the water. The temperature of the water was read at one-minute intervals until five minutes had elapsed. This method, although not very accurate, indicated any decided differences between the two limbs. When we wished to obtain the limb temperatures more accurately at any time, the animal was anæsthetised with ether and "distal thermometers" inserted between the muscles as described above, aseptic precautions being observed. As soon as the temperature reading of the thermometer became constant, the thermometers were withdrawn and the skin brought together by sutures. This method, naturally, could not be used very often.

Exp. 1.—The femoral and sciatic nerves were cut in the right leg of a cat (1·5 kgm.). Five days later the temperature of the denervated paw was decidedly less than that of the normal. Seven days after denervation the animal was anæsthetised and thermometers inserted beneath the gastrocnemii. The temperatures were as follows: Rectal, 38·2° C.; normal limb, 36·7° C.; denervated limb

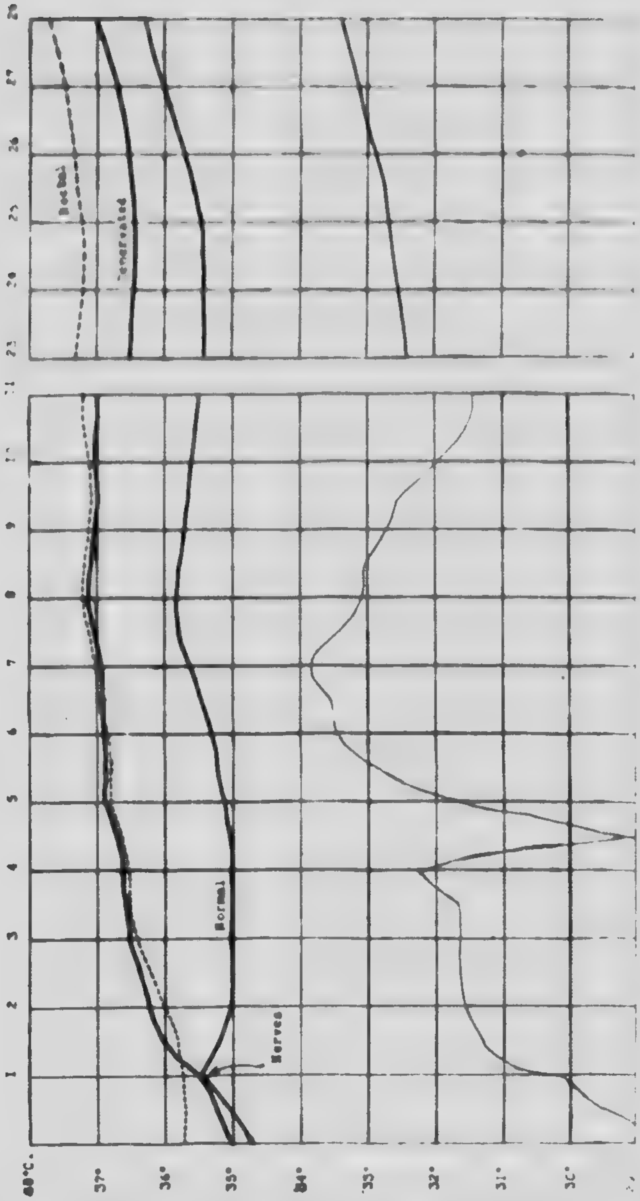

Fig. 5. A comparison of the temperatures of normal and denervated muscles in the hind legs of a cat. Shows partial recovery of the temperature in the denervated limb. Time in hours.

35·92° C. This was unusually rapid recovery, in fact it was "over-recovery," for the operated limb was practically 0·8° C. colder than the normal limb.

Exp. 2.—The right hind limb of a cat was denervated, causing an increased temperature which persisted for something more than two weeks. But in twenty-nine days there had been a decided "over-recovery." On the thirty-third day after the operation the denervated limb was 0·4° C. colder than the normal limb, as determined by thermometers inserted beneath the muscles.

Exp. 3.—In a third cat there was no decided "over-recovery" in eighty-six days. That is to say, the operated limb was slightly warmer (about 0·1° C.) than the control when the animal was deeply under ether, but if allowed to come out of the anæsthetic, the control limb became 0·3° C. warmer. The nerve had regenerated as far as the gastrocnemius (determined by electrical stimulation). There had been marked atrophy of the gastrocnemius. The denervated gastrocnemius-soleus-plantaris group weighed only 4·25 gm. as compared to 15·85 gm. the weight of the control group.

Exp. 4.—A fourth animal showed "over-recovery" of the denervated limb in fifty-six days. This condition persisted for weeks. As late as 151 days after denervation the temperature of the operated limb (thermometers under hamstring muscles) was 1·6° C. lower than the control. However the animal was in poor condition at that time. The nerve had regenerated to the gastrocnemius. It is interesting to note that the veins in the operated limb were much enlarged. The femoral vein was 7 mm. in diameter in the operated limb, while the diameter of the corresponding region of the femoral vein on the control side was 4 mm.

Fibrillation might be in part responsible for the increased temperature which often persists for weeks. Langley and Kato (5) have observed it from the fifth day to as far as 71 days after denervation. Their method was to study the exposed muscle with reflected light. The question occurred to us whether the fibrillation might be due to the stimulation resulting from exposure. We have made the following observations in this connection:

Rabbit. Sciatics cut 15 days before. Exposed gastrocnemius fibrillation immediately. This increased with exposure. Rubbing the surface of the muscle with absorbent cotton increased the fibrillation.

Rabbit. Sciatics cut 28 days before. Fibrillation was apparent in the gastrocnemius as soon as it was exposed. The temperature under this muscle was approximately the same as in the fore limb.

Rabbit. Both gastrocnemii had been denervated 42 days be[...] On exposure of left gastrocnemius, slight fibrillation was immed[...] evident, but this increased considerably during the first two min[...] Similarly the right gastrocnemius showed slight fibrillation imm[...]ately upon exposure, the fibrillation becoming more marked [...] course of the folowing five minutes. The temperature of one of [...] limbs (thermometer inserted beneath gastrocnemius) was 34.7[...] as compared to 36.48° C., the temperature of a normal fore lim[...] the same animal. Fibrillation in the denervated muscle grad[...] disappeared after the animal was killed. It was slightly visib[...] minutes after the heart ceased to beat.

In all cases where fibrillation appeared it was visible immed[...] upon exposure of the muscle, but in many instances it was incre[...] by the exposure. Moreover it has been shown by Schiff (8) [...] fibrillation occurs in unexposed muscle.

Discussion.

What is the cause of the increase in temperature in a dener[...] limb? Heidenhain (3) believed it was due to the increased [...]lation. This is supported by the observation that the in[...] volume of the denervated limb, as we have shown in § I, may [...] for days, as does the temperature. The observation which quest[...] this interpretation is the rapid recovery of the blood vessels [...] denervation, as determined by the venous blood flow (1). Re[...] in such a case being only a matter of minutes.

As stated in § I, the temperature of the air within the plethys[...]graph was often maintained at the high level for hours after the [...] had begun to decrease in volume. It is also difficult to reconcile [...] observation with the explanation that the temperature increas[...] entirely due to circulatory increase. It might be due in part [...] abnormal chemical changes. That abnormal changes are [...] place is evidenced by the later appearance of fibrillation and [...] marked atrophy which occurs.

Every form of skeletal muscle contraction is no doubt ac[...]panied by heat production. Therefore throughout the period [...] paralysis, beginning with the fourth or fifth day after denervation [...] certain amount of heat must be produced attending fibrillation. H[...]ever the quantity generated in this way may be quite small, for dur[...] later stages of paralysis the temperature of the muscle freque[...] becomes markedly lower than that of a normal muscle. Yet we h[...] shown that fibrillation may still be present in such a case.

It is possible that the source of increased heat, which is pre[...] soon after denervation, is a chemical change independent of fib[...]

tion. It must be admitted that such an explanation is merely hypothetical, yet it would reconcile the disagreements of volume change and venous blood in relation to the temperature.

SUMMARY.

Volume changes.

1. The maximum dilatation of the limb occurs from two to six hours after denervation. The extent of this dilatation is probably a little more than 2 p.c. of the total volume.

2. In some individuals, constriction of the denervated limb begins soon after the point of maximum dilatation has been reached. In others, constriction may not begin for a few hours after this time.

3. Complete recovery of the original volume occurs in many cases within twenty-four hours. There may be an "over-recovery" of the original volume as time goes on.

4. Constriction of the denervated limb may take place without a proportionate lowering of the limb temperature.

Temperature changes.

1. The duration of the increased temperature resulting from denervation is exc[illegible]ly variable.

2. In many ca[illegible] ere is an "over-recovery" of the temperature [illegible] occurs from a [illegible]w days to several weeks after de[illegible].

3. Increased circulation and fibrillation do not acc[illegible] tirely for the maintained supernormal temperature of a de[illegible] limb.

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STUDIES IN THE REGENERATION OF DENERVATED MAMMALIAN MUSCLE.

II. EFFECT OF MASSAGE.

By F. A. Hartman, S Sgt., W. E. Blatz and L. G. Kilborn.

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In a recent paper Langley and Hashimoto (1) investigated the effects of massage and electrical treatment in preventing the atrophy of denervated muscle. The atrophy was determined by weighing the excised muscles at the conclusion of the experiment. This method cannot take into account the connective tissue present. In a denervated muscle this tissue becomes proportionately greater as the time is extended. On this account we have attempted to measure the actual power of a muscle to lift a load. This gives an idea of the amount of functioning muscle tissue present. In the present research we have confined ourselves to the study of the effect of massage.

Methods.—The muscles of the lower part of both hind limbs in the rabbit were denervated under aseptic conditions while the animal was under the influence of ether. This was accomplished by either cutting the sciatic nerve and then uniting the cut ends by means of sutures or else by crushing the nerve against a glass rod with a stout thread according to Langley's method.

One of the most difficult problems in working with denervated limbs is their protection against abrasion. This is particularly so in the rabbit because it moves with a hop and when the sciatic is cut the toes are no longer able to take up the shock, which must therefore fall entirely upon the heel. Moreover denervated tissue is more easily injured than normal tissues, due in part, no doubt, to loss of the sensory information from those parts.

We first attempted to protect the heel by soft bandages, but two serious objections presented themselvs; some animals would succeed in loosening the bandages so that they slipped off, or at other times they might be wound so tightly that the foot became swollen. Leather

boots seemed to be an improvement in that they did not come off and did not interfere with the circulation. They were given a thorough trial on a number of animals. Their use had to be abandoned when it was found that many of the rabbits persisted in gnawing great holes in them and sometimes went so far as to eat away a third or a half of the boot. This together with the fact that a few animals persisted in gnawing away their toes suggested the idea of metal boots. After considerable experimentation the following boot was devised.

The boots were made from thin sheet aluminium (Figs. 1 and 2). Movement at the heel was permitted by a brass hinge. The boot was held on the animal by a wire which passed through slits in the back of the upper part and the top of the forward part (*a*, Figs.

Fig. 1. Side view of boot.

1 and 2). The boot was fastened to conform to the resting position of the limb, *i.e.* with the foot flexed. The top piece and the forward part on the bottom and at the edge (*b*) where rubbing might occur were lined with flannel. Ventilation was afforded by holes in the sides and at the front. This was necessary in hot weather. As an additional safeguard absorbent cotton was placed under the heel and sometimes where the upper edge of the forward part of the boot (*b*) might rub. Wherever the hair was worn away or the skin became chafed, collodion was applied.

Massage was given by volunteer masseuses from the School of Massage, Hart House. In all cases the right leg was massaged by

Fig. 2. Top view of boot.

the methods considered most appropriate for small muscles, viz., a gentle kneading and stroking. Both the left and right legs were put through passive movements three times to prevent stiffening of the joints. Such treatment was given from five to six days per week.

At the termination of the experiment, the rabbit was anæsthetised with urethane administered by a stomach tube; the muscles which have their insertion in the tendon of Achilles were carefully dissected out so as to disturb their circulation as little as possible; and then the animal was placed belly downwards on an animal board so that the hind limbs extended beyond the board. The tibia was held firmly by a clamp in such a way that the group of muscles (gastrocnemius, soleus and plantaris) to be tested hung freely as soon as the tendon of Achilles was cut. Drying of the muscle was prevented by frequent application of Ringer's solution or by smearing the outer surface with vaseline. The latter method seemed to give just as good results as the former.

The tendon was fastened to a lever from which at the same point was suspended a scale pan (Fig. 3). Both limbs were prepared at

the same time in the same way so that simultaneous records could be obtained of the corresponding groups of muscles on the treated and untreated side.

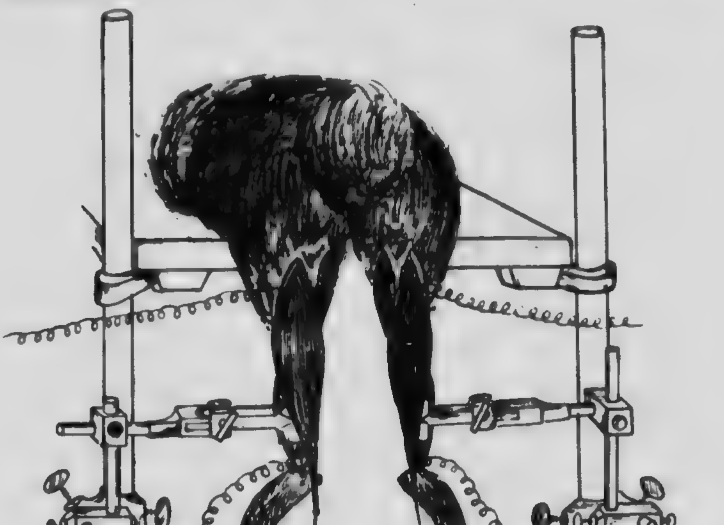

Fig. 3. Apparatus for testing muscles.

The muscle was stimulated by either induced or direct currents. In a majority of cases the induced current was used. When the former was used, one fine wire was fastened to the tendon and another above the origin of the muscles around bone and muscle. In the case of the direct current, cotton strips soaked in Ringer's solution connected the above described regions of the muscle with non-polarisable boot electrodes. The muscle was after-loaded. Care was taken to insure equal initial tension for the two muscle groups. The strength of current necessary to produce maximum contraction was employed. A series of contractions from gradually increased weights was obtained from each muscle group. In order to rule out variations resulting from circulatory changes due to ether anæsthesia, either simultaneous contractions of the two groups were secured or else the contraction of one group immediately followed the contraction of the other group for each increment. When the rabbits were placed under the influence of urethane the fluctuation due to anæsthesia did not occur.

Thus a series of contractions, with loads increasing by a definite increment, as 2 gm., 4 gm., or 10 gm., depending upon the power of the muscle, was obtained for the treated and untreated denervated muscles under similar conditions. The load often went as high as the absolute limit. In order to see whether fatigue in any way altered the relative power between the muscles of the two sides as many as six sets of records were made in many tests. From these a typical set was chosen for plotting the work curve.

As soon as the functional tests of the muscles were completed, the muscles on each side were carefully removed and weighed with the observation of such precautions as similar dissection and prevention of drying (*see* Langley (1), p. 16). The muscles were then fixed in Orth's solution for histological study.

Load and work curves were plotted from all records obtained. The curve for the treated muscle group was drawn on the same sheet with that for the untreated muscle group. Thus corresponding parts of the curves could be compared at a glance.

Results.—An indication of the variation between the right and left gastrocnemius-soleus-plantaris group was obtained from a study of fifteen normal rabbits (Table I). A comparison of the work performed at the optimum load gives a fair idea of the two curves for comparison. It is to be noted, too, that the heavier muscles do not always have the greater capacity for work, although there is a surprising tendency in that direction. Moreover the left muscles were stronger than the right in nine of the fifteen animals. Treated animals in which sores developed were discarded if the muscles were affected in any way.

The percentage difference between the corresponding mu groups varied so greatly that to strike an average would mean lit In view of this and not knowing what the muscle could do bef denervation it would be necessary to obtain a very marked incre of power in the treated muscle as compared to the control to p that massage was of value.

The duration of the massage was increased from week to week many cases, as the table indicates. It being assumed that massage might be needed more as the circulation decreased. T principal indication that the circulation was decreased, being lowered temperature in long-standing denervations.

Thirty-seven animals were successfully carried through to com pletion. The duration of the treatments ranged from seven to 190 days. Twenty-three of these possessed stronger muscles on the trea side, the remaining fourten having stronger muscles on the untrea side. In th animals in which the treated muscles were stron they average 64·2 p.c. greater than the control muscles, while in th rabbits possessing more powerful control muscles, the average incre over the treated muscles was 43·7 p.c.

TABLE I. A comparison of weights and work performed at the optimum l in normal gastrocnemius-soleus-plantaris muscle groups.

Animal	Work at optimum load in gm.-mm.		Percentage difference, right referred to left.	Weight in grams.		Percenta difference
	Right.	Left.		Right group.	Left group.	
38	82	79	− 2			
39	128	202	− 37	8·46	8·86	4 [illegible]
40	72	117	− 38	9·16	9·61	− 4·7
41	65	67	− 2·9	11·61	12·06	3·7
42	158	203	− 22	9·46	9·66	2·07
43	102	147	− 30·6	9·7	9·8	− 1·04
44	263	240	9·6	9·30	9·01	3·2
45	60	78	− 23	11·58	11·66	− [illegible]
46	79	65	21·6	10·23	10·45	− 2·1
47	65	179	− 63·6	16·10	15·76	2·[illegible]
48	77	64	20	12·31	11·83	4·05
49	84	157	− 46·5	14·70	15·06	− 2·4
50	88	78	20	14·05	14·13	− [illegible]
51	63	59	6·7	13·60	13·91	2·2
52	194	156	24	17·26	17·03	13·[illegible]

The normal muscles of Table I showed a predominance of over right, not only in the number of animals involved, but in percentage of differences. Where the left muscle group was greater power, the average was 29·5 p.c. over the right, while in the cases right preponderance the muscles averaged only 15·9 p.c. more po

ui than the left. If it should turn out to be true that rabbits, on the average, possess more powerful muscles in the left leg than in the right, that would strengthen the observations in Table II and indi

TABLE II. Comparison of treated and untreated denervated muscle.

Animal	Duration of massage each day in min.*	Duration of treatment in days.	Work at optimum load in gm.-mm. Right group (massaged.)	Work at optimum load in gm.-mm. Left group (control.)	Percentage difference, right referred to left.	Weight in grams. Right group	Weight in grams. Left group	Percentage difference, right referred to left
1	3	7	59	29	104	9·03	9·06	0
2	3	11	66	43	53	9·63	9·65	0
3	2	13	24	17	41	7·81	7·81	0
4	3, 4	14	111	202	− 62	7·63	8·25	− 8
5	30	15	130	152	− 15	6·40	7·79	−18
6	3, 4	17	260	188	37	7·38	7·28	2
7	3, 4	20	287	112	156	9·26	8·80	5
8	30	20	272	334	− 19	5·3	5·2	2
9	3, 4	25	147	135	9	7·11	7·91	−11
10	3, 4, 5, 6	26	137	54	154	....	....	
11	3, 4	29	290	181	60	7·67	7·11	....
12	3, 4, 5, 6, 7	30	187	129	45	6·29	5·86	7
13	3, 4, 5, 6	34	133	189	− 42	5·06	5·48	− 8
14	3, 4	36	104	224	− 54	4·7	4·65	2
15	3, 4	38	54	47	15	7·51	8·18	− 9
16	3, 4	40	57	98	− 45	6·43	6·91	− 8
17	2,	41	87	59	47	4·85	4·80	0
18	3, 4	44	273	190	44	6·2	5·7	9
19	2, 3, 4, 5	44	190	263	− 28	4·10	4·12	0
20	2, 3, 4, 5, 6	45	184	128	44	6·4	5·45	18
21	3, 4	45	158	251	− 37	6·8	6·6	3
22	2, 3, 4	48	40	13	208	4·23	3·94	8
23	2, 3	49	38	28	36	5·1	4·2	21
24	3, 4, 5, 6, 7	50	37	30	26	....	....	....
25	3, 4, 5, 6, 7, 8, 9, 10	61	140	52	169	7·86	7·82	1·4
26	3, 4, 5, 6, 7, 8	65	141	107	32	7·30	7·87	− 8
27	3, 4, 5, 6, 7, 8	68	80	83	− 4	7·56	8·71	15
28	3, 4, 5, 6, 7, 8, 9, 10	78	38	56	− 44	5·44	5·30	3
29	3, 4, 5, 6, 7, 8, 9, 10	81	34	71	−109	5·80	5·10	14
30	3, 4, 5, 6, 7, 8, 9	83	111	49	123	6·64	6·87	− 3
31	2, 3, 4	91	258	250	34	6·5	6·5	0
32	3, 4, 5, 6, 7, 8, 9, 10	93	123	287	−133	5·79	7·41	−28
33	3, 4, 5, 6, 7, 8, 9, 10	164	98	76	29	9·55	8·56	11
34	3, 4, 5	105	136	100	36	8·39	8·48	− 2
35	3, 4, 5, 6, 7, 8, 9	152	157	183	− 14	11·5	12·5	− 8
36	3, 4, 5, 6, 7, 8, 9	190	234	216	8	12·50	12·20	2
37	3, 4, 5, 6, 7, 8, 9	190	344	361	− 6	14·6	15·4	7

* Where a series of numbers is given, it means the number of minutes each succeeding week, the treatment being maintained at the last figure for the balance of the time.

† Treated 30 days, then without treatment for 20 days before testing.

‡ Nerve regenerated as determined by stimulation or by voluntary movement of toes.

§ Conduction in right tibial nerve, but not in left.

cate some benefit from massage. However the number of animals used in Table I was too small to establish such a generalisation.

A very important consideration must be kept in mind, and that is concerning the atrophy of denervated muscle. Such a muscle dwindles in volume and no doubt in power, so that if the massaged muscle is compared with the corresponding untreated muscle by its capacity to do work, an increase of 64 p.c. over the control does not mean so much as it would in normal muscles. In other words both treated and untreated muscles dwindle rapidly, but the massaged muscle maintained 64 p.c. more strength. The treated muscles might lose a great deal and still do that. Even this might be of more significance if a large majority of the animals treated showed this, but this is true in only twenty-three out of thirty-seven animals treated. Slightly more than a third of the number showed an opposite condition.

Judged from the functional test, therefore, massage has not proven to be of great value in our experiments. But because we did not know the relative capacities of the muscles before denervation, we cannot make our conclusions as positive as might be done otherwise. Moreover the method of testing is open to the objection that there is some disturbance of the circulation in the preparation of the muscle so that its tendon can be fastened to the lever. Although the two sets of muscles were prepared in as similar a manner and as quickly as possible, a certain amount of disturbance in the muscle was unavoidable.

There are certain observations which we will point out in reference to the comparative weights of treated and untreated muscles. In the first place the percentage difference in weight never reaches the range attained in the functional test. In the second place if the functional test shows a much greater preponderance of one muscle group over the other that muscle group frequently weighs less than the other. All told, out of the thirty-seven experiments, seventeen did not agree in functional and weight tests as to which preponderated.

These observations seem to indicate that the weight of a muscle does not necessarily indicate the relative amount of contractile tissue.

In conclusion we wish to thank Miss Joan Campbell, who gave valuable assistance in this research. We wish also to thank the members of the Schol of Massage at Hart House for their part in the research.

Summary.

1. The soleus, gastrocnemius and plantaris muscles were denervated on both sides in thirty-seven rabbits. The muscles of the right side were massaged from two to ten minutes a day over periods varying from seven to 100 days. At the conclusion of the experiment the work capacity of the treated muscles was compared with that of the control. The two groups of muscles were also weighed.

2. In a similar manner the same muscles in fifteen normal animals were compared on both sides.

3. Sixty per cent of the normal rabbits possessed stronger muscles on the left side. In the cases of left preponderance the difference was much greater than in the cases of right preponderance. A large proportion of the left muscle groups were also heavier than the right, although such muscles did not invariably prove to be the stronger.

4. The massaged muscles were stronger than the controls in sixty-two per cent of the animals treated. There was considerable discrepancy between the comparison by weight and the comparison by function.

5. In view of our ignorance of the relative capacities of the two sets of muscles before beginning treatment, a small predominance of power in the treated muscles is inconclusive. Our observations indicate that massage of denervated muscle is slightly beneficial[1].

REFERENCES.

(1) Langley and Hashimoto. Brit. Jour. Physiol. 52. 199. 1899.
(2) Ricker. Arch. Path. Anat. und Physiol. 158. 15. 1918.

[1] A subsequent study upon the effect of massage on denervated muscle, involving a larger number of animals and a method of testing the muscle functionally both before operation and during the period of treatment will appear later.

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